Nuclear chromatin texture and sensitivity to DNase I in human leukaemic CEM cells incubated with nanomolar okadaic acid

S. Yatouji*, F. Liautaud-Roger† and J. Dufer*

*Unité Médian, Faculté de Pharmacie, Université de Reims, Reims, France, and †Institut Jean-Godinot, Reims, France

(Received 26 January 1999; revision accepted 6 October 1999)

Abstract. It is now known that the analysis of chromatin texture can be used in oncology as a sensitive detection method, either to define diagnostic classifications or to locate a lesion along a defined trend curve. However, the functional significance of these variations in textural features remains sometimes unclear. Several drugs have been shown to be able to modulate chromatin structure. Among them, the phosphatase inhibitor okadaic acid at low concentration can increase accessibility to DNA in chromatin of carcinoma cells. This paper demonstrates that short exposures (0–3 h) to a 10-nM dose of okadaic acid induced an increased sensitivity to DNase I digestion in human CEM leukaemic cell nuclei and that this sensitization was associated to variations of nuclear texture characteristics, as evaluated by image cytometry. CEM cells treated with okadaic acid for 0–3 h displayed changes in chromatin supraorganization with a more homogeneous and fine chromatin texture, as compared to control cells. This suggests that the appearance of an open configuration of chromatin structure as evaluated by biochemical methods corresponds to a more decondensed texture of nuclei measured by image cytometry. Longer exposures (6–24 h) of CEM cells to 10 nM okadaic acid lead to apoptosis. As reported previously for camptothecin-treated HL60 cells, okadaic acid-treated CEM cells display biphasic nuclear chromatin texture changes, i.e. a decondensation phase followed by the appearance of typical apoptotic cells with a smaller nuclear area and a highly condensed chromatin. Finally, using the multidrug-resistant CEM-VLB cell line, it was confirmed that these multidrug-resistant cells also display cross-resistance to okadaic acid, as this compound was unable to induce either increased DNase I sensitivity, apoptosis, or altered nuclear texture in this particular cell line.

INTRODUCTION

Various cytological processes such as cell proliferation, differentiation, transformation, apoptosis etc., are accompanied by chromatin changes, usually identified on the basis of the

Correspondence: Professor J. Dufer, Laboratoire de Physiologie Cellulaire, Unité Médian, EA 2063, Faculté de Pharmacie, 51, rue Cognacq-Jay, F-51100 Reims Cedex, France.
E-mail: jean.dufer@univ-reims.fr

distribution of euchromatin and heterochromatin within the nucleus. Descriptions of chromatin patterns typically include such terms as 'fine, coarse, clumped, pale...' and the subjectivity of this process precludes reproducibility. One way to achieve a quantitative, non-subjective evaluation of the chromatin pattern consists in the analysis of the chromatin texture by image cytometry (for review, see Doudkine *et al.* 1995). Texture features can be roughly classified into categories as descriptive statistics of chromatin distribution, discrete texture features, range extreme, Markovian run length, and fractal features (Doudkine *et al.* 1995).

These features have proven to have value in the description of nuclei both in physiological and in pathological cell states, and in tumours, dramatic changes in the nuclear chromatin appearance are common and have been associated with the progression of the disease (Deligdisch *et al.* 1993, Doudkine *et al.* 1995, Bartels *et al.* 1998). However, as it is still difficult to establish a close correlation between the variation of textural parameters and the eventual visible changes in chromatin, current texture parameters appear abstract, sometimes hamper understanding, or lead to cryptic results. Moreover, the functional significance of the variations in textural features is sometimes unclear. For example, it has been proposed that changes in chromatin condensation supraorganization could be related to proliferation state (Santisteban & Brugal 1995), transcription activities in cell nuclei (Vidal, Russo & Mello 1998), DNA synthesis rate (Dörmer & Abmayr 1979), chromosomal rearrangements (Liautaud-Roger *et al.* 1992), or activation patterns of genes (Mello *et al.* 1995, Fischer *et al.* 1998).

A recent prior study showed that apoptosis induced by a topoisomerase I inhibitor was associated with an early textural 'decondensation' of nuclei and this was attributed to the appearance of a more open configuration of the chromatin structure (Palissot *et al.* 1996b). Several drugs are able to modulate chromatin supraorganization. Among them, the phosphatase inhibitor okadaic acid at low concentrations can increase accessibility to DNA in chromatin (Rieber & Rieber 1992). This paper examines the consequences of this increased DNA accessibility on textural features as measured by image cytometry.

MATERIAL AND METHODS

Chemicals

Okadaic acid (OA) was obtained from Sigma Chemical Co (St Louis, MO, USA) and was dissolved (25 µg/ml) in dimethylsulphoxide (DMSO). Cell culture RPMI 1640 medium and fetal calf serum were purchased from Gibco/BRL (Grand Island, NY, USA). Stock preparations from all reagents were stored at -20°C. All were diluted to appropriate final concentrations in medium.

Cell culture

The human lymphoblastic leukaemia CCRF-CEM cell line and its multidrug-resistant variant CEM/VLB100 were obtained from W.T. Beck, Memphis, TN, USA and Z. Steplewski, Philadelphia, PA, USA respectively. These were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 20 µM L-glutamine, 100 units/ml penicillin G, and 100 µg/ml streptomycin. All cultures were maintained under a fully humidified atmosphere of 95% air - 5% CO₂ at 37°C. CEM-VLB were grown in the presence of 0.4 µg/ml vinblastin. All cells were passaged twice weekly and all experiments with resistant cells were performed on cells cultured in drug-free medium for more than 48 h. Exponentially

growing cells were used in all experiments. Cell viability was assessed by the ability to exclude trypan blue (0.5% w/v, Sigma).

Treatment with okadaic acid

Cells were seeded at 2×10^5 cells/ml in medium with okadaic acid at 10 nM. Cells were then returned to a 37°C, 5% CO₂ incubator until required for analysis. Okadaic acid stock solution was diluted to ensure a final concentration of less than 0.03% DMSO. Control cultures were treated with an equivalent volume of DMSO in RPMI. Cell growth was assessed by the MTS/PMS colorimetric assay (Promega, Madison, WI, USA). Apoptosis induction was monitored morphologically as described by Roger *et al.* (1996) after staining of the cells with a mixture of acridine orange (Sigma, 100 µg/ml) and ethidium bromide (100 µg/ml) in phosphate-buffered saline (PBS), and immediate examination under a fluorescence microscope (Zeiss, Strasbourg, France) at a 490-nm excitation wavelength.

Sensitivity to DNase I

Cells were washed twice in PBS and resuspended in PBS to a final concentration of 10^7 cells/ml. One volume of cell suspension was mixed with one volume of ice cold lysis buffer (320 mM sucrose, 5 mM MgCl₂, 10 mM Tris-HCl, 1% Triton X100, pH 7.5), 3 volumes of ice cold distilled water, and incubated for 10 min on ice. After centrifugation (1300 g, 15 min, 4°C), the pelleted nuclei were suspended at 10^7 /ml in 0.5 ml lysis buffer.

Fifty microlitres of DNase I (Boehringer Mannheim, Germany) solution (100 units/ml) in incubation buffer (20 mM Tris-HCl, pH 7, 1 M NaCl, 10 mM MnCl₂) were added and incubated for 10 min at 37°C. After centrifugation, nuclei were lysed in 5 ml lysis buffer (800 mM GuHCl, 30 mM EDTA, 30 mM Tris-HCl, 5% Tween-20, 0.5% Triton, pH 8) containing 95 µl protease solution (20 mg/ml, Qiagen, Hilden, Germany) for 1 h at 50°C.

Genomic DNA was purified on anion-exchange resin according to the manufacturer's protocol (Qiagen). DNA was then incubated during 2 h at 37°C with 0.1 µg/ml RNase (Dnase-free) (Boehringer) prior to electrophoresis on 1% (w/v) agarose gel for 90 min at 80 V. Equal amounts of DNA (5 µg) were loaded in each lane as determined by absorbance measurement at 260 nm; 1 µg lambda/HindIII molecular weight marker (23130–564 bp, Gibco) was run in parallel. Electrophoregrams were scanned using an image analysis system (Vilbert Lourmat, Marne la Vallée, France) and analysed on a Macintosh 6100/60 using the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>).

Image cytometry

Cells were smeared by cytocentrifugation at 100 g for 5 min, air-dried and fixed in alcohol-formalin mixture (95% ethanol, 3% formaldehyde in saline; 3:1) for 10 min. After acid hydrolysis with 5M HCl for 30 min, they were stained by the Feulgen method as previously described (Palissot *et al.* 1996a). Image cytometry was performed with an image analysis system (SAMBA 2005, Unilog, Grenoble, France) coupled to a colour three CCD camera (XC-007P, Sony Corporation, Japan) and a microscope (Axioskop, Karl Zeiss, Oberkochen, Germany). Measurements were made with a plan-achromat ×40 objective. After mitoses counting, at least 200 interphase cell nuclei were analysed on each microscopic slide. Eleven parameters were computed from each nuclear image: one geometric feature (nuclear area, NA), one densitometric parameter (integrated optical density, IOD), and nine textural parameters. The IOD is the summation of all pixels in the image of nucleus and quantitatively measures the total intensity of staining by the DNA-

Table 1 Computed textural parameters

Co-occurrence matrix		
Local mean	LM	Gives a global estimation of chromatin condensation. Increases with condensation.
Energy	E	Express the chromatin distribution rather than its condensation. Decreases with compact distributions.
Entropy	ENT	Both parameters measure the contrast in an image. Increase with contrast.
Inertia	I	
Run-Lengths matrix		
Short run-lengths emphasis	SRE	These parameters measure the distribution of run-lengths that depend on the chromatin organization. LRE increases and SRE decreases when the chromatin texture is finely granular and homogeneous. Inverse variations when texture is heterogeneous with large clumps.
Long run-lengths emphasis	LRE	
Grey-levels distribution	GLD	Measures the uniformity of grey-levels distribution among the run-lengths
Run-lengths distribution	RLD	This parameter increases with the homogeneity of run-lengths.
Run-lengths percentage	RPC	This parameter leads to interpretations similar to SRE.

For details see Giroud (1987).

binding dye. Since DNA staining with Feulgen reaction was stoichiometric, IOD was directly related to total nuclear DNA content. The distribution of nuclei according to IOD was plotted to provide cell cycle distribution. The margins defining G_0/G_1 and G_2 peaks were defined as modal IOD $\pm 15\%$ (Dufer *et al.* 1995). At least 100 reference cells (mouse hepatocytes or human lymphocytes) were measured in the same conditions for the calibration of the normal diploid (2c) value.

Textural features estimated by image cytometry are closely related to the location of a given cell in the cell cycle. Comparisons of textures should therefore be performed on cells selected within a given cycle phase. For this purpose, G_0/G_1 cells were isolated on the basis of their DNA–IOD value, and analysed for chromatin texture. Although they could display slightly different nuclear structures, G_0 cells were not analysed separately, as they represent a minor component of such cell populations in growth phase (less than 1% as evaluated by Ki-67 staining, i.e. below the resolution of the analysis system) (Gorisse *et al.* 1990). Nine textural parameters were calculated on the nuclear image after reduction to 16 grey levels by linear rescaling (Giroud 1987). Four features were calculated on the grey-levels co-occurrence matrix: Local mean (LM), energy (E), entropy (ENT), and inertia (I). Five parameters were calculated on the run-length matrix: short run-length emphasis (SRE), long run-length emphasis (LRE), grey levels distribution (GLD), run-length distribution (RLD), and run-length percentage (RPC) (Haralick, Shanmugan & Dinstein 1973, Galloway 1975, Giroud 1987). A brief description of these parameters appears in Table 1.

Data analysis and statistics

The distribution, mean, and SD of the nuclear parameters were calculated for each cell population. Significance of the differences between parameters values was estimated by one-way analysis of variance. Multivariate analysis was performed using the SAMBA software (modified BMD program — BMD07M, University of California, Los Angeles, CA, USA). Classification by discriminant analysis was based on use of the *F*-test to select variables. The classification rates of the cells into predicted groups were estimated by splitting the data sets

Figure 1. Growth of CEM and CEM-VLB cells cultured for 3 days in the presence or absence of 10 nM okadaic acid (OA). Solid circles: CEM cells without OA; solid squares: CEM cells with 10 nM OA; open circles: CEM-VLB without OA; open squares: CEM-VLB with 10 nM OA.

into two equal parts, defined as learning and test sets. In these estimations, discriminant functions were obtained on learning groups only and test groups were classified on these bases. Canonical analysis was used to display the differences between groups. Canonical variables were calculated on learning sets and cells from test groups projected as 95% confidence ellipses around the mean cell profiles onto the plane defined by the two first canonical variables.

RESULTS

Cell growth changes induced by okadaic acid

Effects of 10 nM OA on cell growth and the cell cycle of CEM and CEM-VLB cells were examined. The toxin was found to inhibit the cell growth of CEM cells markedly, whereas CEM-VLB cells remain unaffected (Figure 1). Lower doses (2.5 and 5 nM) were assayed in pilot experiments, but did not lead to significant growth or nuclear texture changes (data not shown). Cell cycle phases were extracted by isolation of G_0/G_1 and G_2 peaks on image cytometry data and counting mitoses on the same slides. Short exposures to OA (0–3 h) did not affect cell cycle distributions either in CEM and CEM-VLB cells. On the contrary, after 12 and 24 h of treatment, CEM cells displayed a significant increase in mitotic cells and G_2 cells (67% of interphase cells after 24 h) with a concomitant appearance of typical apoptotic cells as detected by the acridine orange–ethidium bromide method (Table 2).

DNase I digestion

Nuclei were isolated from CEM and CEM-VLB cells either treated or not for 2 h with 10 nM OA. The nuclei were then digested by DNase I, and the digestion products were

Table 2 Effects of okadaic acid treatment (10 nM) on cell cycle distribution of CEM cells.

Cell population	Incubation time (h)	G ₀ G ₁ (%)	S (%)	G ₂ (%)	Mitoses (%)	Apoptotic cells (%)
CCRF-CEM	0	50.0 ± 1.0	21.0 ± 1.5	24.6 ± 2.3	4.4 ± 0.4	0
	1	48.6 ± 7.5	21.6 ± 4.1	25.1 ± 5.3	4.7 ± 0.5	0
	2	47.3 ± 9.2	23.7 ± 6.1	24.2 ± 3.5	4.8 ± 0.4	0.5
	3	53.8 ± 2.0	23.4 ± 4.5	16.4 ± 6.4	6.4 ± 2.2	1
	6	50.1 ± 3.4	24.1 ± 5.2	20.5 ± 4.8	5.3 ± 0.9	3
	12	38.2 ± 5.2	25.1 ± 4.7	21.1 ± 4.3	15.6 ± 1.4*	4.9
	24	6.6 ± 1.0	11.8 ± 0.5	37.2 ± 1.1	64.4 ± 14.6*	13.9
CEM-VLB	0	56.9 ± 2.2	15.0 ± 4.1	24.6 ± 4.6	3.5 ± 0.6	0
	1	54.3 ± 4.7	22.1 ± 0.6	20.1 ± 4.1	3.5 ± 1.4	0
	2	50.9 ± 3.4	20.0 ± 4.5	25.6 ± 4.4	3.5 ± 0.6	0.1
	3	55.0 ± 2.5	22.3 ± 1.1	18.3 ± 1.4	4.4 ± 2.2	0.05
	6	50.3 ± 4.2	21.1 ± 3.6	23.3 ± 2.9	5.1 ± 0.9	0.09
	12	51.5 ± 4.4	18.2 ± 3.1	25.5 ± 4.1	4.8 ± 1.6	1.7
	24	50.8 ± 5.9	23.0 ± 5.4	21.3 ± 1.9	4.9 ± 1.2	1.4

DNA content was estimated by measuring integrated optical density (IOD) on Feulgen-stained interphase nuclei. Cell cycle estimations were computed on these DNA histograms as previously reported (Dufer *et al.* 1995). Mitotic figures were counted on the same slides. Apoptotic cell percentages were evaluated on separate samples after acridine orange ethidium bromide staining. Each figure represents mean ± SD from 3 separate experiments. * $P < 0.05$ as compared to distribution in untreated controls (χ^2 test).

electrophoresed on agarose gels. The ethidium bromide stained gel (Figure 2) demonstrates that in CEM cells with 5 units DNase I a smear pattern of DNA fragmentation was produced. However, no clear-cut internucleosomal DNA cleavage was produced. In OA-treated CEM nuclei, the DNA appears more sensitive to digestion. When assessed by scanning of DNA smears, the increase in smear integrated density in OA-treated cells vs. untreated cells is 40.5%. In contrast, treatment with OA did not increase DNase I sensitivity in CEM-VLB cells.

Analysis of nuclear texture

Values of the nuclear area and nine textural features were estimated in G₀/G₁ cells during a 24-h incubation of CEM and CEM-VLB cells with 10 nM OA and compared with values

Figure 2. DNase I sensitivity of nuclei from CEM and CEM-VLB cells treated with 5 units of DNase I with or without pretreatment with 10 nM OA for 2 h. Lane 1: molecular weights control; lane 2: CEM cells + OA; lane 3: CEM cells alone; lane 4: CEM cells + OA + DNase I; lane 5: CEM cells + DNase I; lane 6: CEM-VLB cells + OA; lane 7: CEM-VLB cells alone; lane 8: CEM-VLB cells + OA + DNase I; lane 9: CEM-VLB + DNase I. This figure shows a representative experiment from a series of three.

Figure 3. Variations of some representative cytometric parameters in CEM and CEM-VLB cells treated with 10 nM OA for 0–24 h. Each point represents mean $\pm$ SEM of data from three independent experiments. Solid circles and solid line: CEM cells; open circles and dotted line: CEM-VLB cells.

observed in the respective control cell populations. Among these features, four (LM, E, SRE, and NA) were selected on the bases of their discriminatory power between the cell populations in a linear discriminant analysis, and of their mutual independence. The variations of these parameters are shown in Figure 3. In CEM cells, short exposures to OA (0–3 h) induced a progressive and significant decrease in SRE (which corresponds to the

appearance of a more fine and homogeneous texture) and an increase in E (underlining a different distribution of chromatin), without changes in LM (which gives a global estimate of the condensation level), or NA. After 6–24 h of treatment, CEM cells displayed an increase in chromatin condensation, as evidenced by the increase in LM and SRE, and the decrease in NA. On the contrary, OA treatment did not induce any change in textural parameters values measured on CEM-VLB cells. These changes could be further visualized after a canonical analysis of the data observed on the nine textural features. The representation of the experimental groups onto the two-dimensional plane resulting from this canonical analysis is shown on Figure 4. The first canonical variable (CV1) explained 87.5% of the variance between the groups and mainly allowed separation of cell types. This representation shows that the nuclear texture of sensitive cells nuclear texture displays biphasic changes: decondensation and spatial reorganization of a more homogeneous chromatin (increase of LRE and E) from 0 to 3 h, followed after 6–24 h by condensation (increase in SRE, LM and RPC), whereas resistant cells nuclei do not display any significant alteration.

DISCUSSION

The present study has shown that short exposures to a nanomolar dose of OA induced an increased sensitivity to DNase I in human CEM leukaemic nuclei and that this sensitization was associated to variations of nuclear texture characteristics. OA-treated CEM cells for 0–3 h displayed a more homogeneous and fine chromatin texture than control cells. This suggests that the appearance of an open configuration of chromatin structure as evaluated by biochemical methods corresponds to a more decondensed texture of nuclei measured by image cytometry. It has been postulated that chromatin texture could be described in terms of condensation (compactness of chromatin segments), distribution (relative proportions of compactness levels), and organization (topographic arrangements of condensation degrees) (Giroud 1987). It is interesting to note that the OA-induced textural changes concerned mainly SRE and E, two features related to the spatial organization and distribution of chromatin, respectively, rather than to decondensation *per se* (Giroud 1987). LM, a feature giving a global estimate of the whole chromatin condensation, appeared less modified, as did the nuclear area.

From a pathological point of view, it is now acknowledged that the analysis of chromatin texture can be used in oncology as a sensitive detection method, either to define diagnostic classifications or to locate a lesion along a defined trend curve (Bartels *et al.* 1998). For example, such texture analyses have been applied to classifications of tumours from breast (Dufer *et al.* 1993, Weyn *et al.* 1998), bladder (van Velthoven *et al.* 1995), ovary (Deligdisch *et al.* 1993), prostate (Jorgensen *et al.* 1996), and colorectal cancer (Hamilton *et al.* 1995), as well as to the description of individual tumours by definition of textural signatures (Bartels *et al.* 1998).

The biological significance of these changes in nuclear texture appears sometimes unclear and could be often associated with multifactorial causes. It has been shown that nuclear texture was associated with the rates of synthesis of DNA (Dörmer & Abmayr 1979) and proteins (Dörmer, Abmayr & Giaretti 1984). Textural changes have also been described during cell differentiation (Giroud *et al.* 1988, Dufer *et al.* 1989), or proliferation (Giroud *et al.* 1988, Santisteban & Brugal 1995). Moreover, texture alterations can be linked to chromosomal abnormalities (Liautaud-Roger *et al.* 1992) and oncogene activities (Mello *et al.* 1995), and may be influenced by exogenous stimuli such as drug effects (Colomb, Dussert & Martin 1991, Vidal *et al.* 1998).

Figure 4. Canonical analysis of cytometric data obtained from textural features. Canonical plane was computed and 95% confidence ellipses around the mean cell profile of each population were projected onto this plane. Panel (a): CEM cells; panel (b): CEM-VLB cells; panel (c): feature vectors. The length and the direction of a vector is directly related to the strength of the corresponding feature in the calculation of the canonical variable. Only the five most significant features are displayed.

In this work, it was showed that the induction of chromatin structure relaxation by a short OA treatment was actually associated with texture changes. These texture changes occurred between 1 and 3 h OA incubation. These modifications could not be ascribed to different percentages of cells in G_1 , S, or G_2 phases in the population analysed. Indeed, treatment with OA for such periods did not change the cell cycle phase distribution of

nuclei (Table 2). Moreover, texture analyses were performed on G₀/G₁ selected cells. Such an increase in DNase I sensitivity was previously reported on A431 carcinoma cells treated for 90 min by 10 nM OA (Rieber & Rieber 1992). At this concentration, OA inhibits phosphatase 2A, whereas it inhibits phosphatases I and 2B at higher levels (Schonthal 1995). The mechanisms involved in these chromatin alterations remain controversial. These changes are reflected by a greater endonuclease susceptibility in the chromatin treated with OA correlated with a decrease in a 80-kDa AT-rich DNA binding protein and an apparent hyperphosphorylation of a 70-kDa nuclear matrix protein (Rieber & Rieber 1992). Histone phosphorylation (H1, H2A) and acetylation (H3) levels could modulate chromatin condensation (Chadee *et al.* 1995, Taylor *et al.* 1995). Pre-treatment of cells with phosphatase inhibitors *p*-(chloromercuri)-benzenesulphonic acid (CMBS) or OA both lead to an enhanced DNase I sensitivity and to histone H2A phosphorylation (Feng *et al.* 1991, Rieber & Rieber 1992, Ajiro *et al.* 1996). However, other mechanisms, such as oncogene levels, could be implicated as well. Cells expressing various oncogenes displayed increased histone phosphorylation and a relaxed chromatin structure (Chadee *et al.* 1995). Moreover, transfection of CHO cells with mutant p53, HPV 16-E6, or cyclin G transgenes results in the disruption of higher order chromatin structure (Smith *et al.* 1998).

The consequences of these changes in chromatin supraorganization might be of importance. Longer exposures of CEM cells to 10 nM AO lead to mitotic arrest and apoptosis (Table 1), two phenomena that have been shown to result from independent mechanisms (Lerga *et al.* 1999). As reported previously for camptothecin-treated HL60 cells (Palissot *et al.* 1996b), AO-treated CEM cells display biphasic nuclear chromatin texture changes, i.e. a decondensation phase followed by the appearance of typical apoptotic cells with a smaller nuclear area and a highly condensed chromatin. Previous studies indicate that a decondensation of chromatin would be a prerequisite change which permits subsequent access to the chromatin by factors involved in DNA replication or transcription (Chadee *et al.* 1995), or apoptosis (Ferlini *et al.* 1996, Palissot *et al.* 1996b). Indeed, the apoptotic pathways induced by the two drugs could be different. For example, the caspase I selective protease inhibitor YVAD-cmk inhibited camptothecin-induced morphology, but not OA-induced morphology (Jensen *et al.* 1999). However, it must be stressed that incubation of cells for 24 h with a low dose of OA leads to apoptosis with *bcl2*-downregulation (Riordan *et al.* 1998), a phenomenon also observed with camptothecin (Palissot *et al.* 1998).

Finally, using a previously untested cell line, the present study confirmed that CEM-VLB multidrug-resistant cells also display cross-resistance to OA. OA was unable to induce either increased DNase I sensitivity, apoptosis, or altered nuclear texture. These cells therefore could represent a convenient negative control for experiments involving OA treatments. The mechanisms responsible for this cross-resistance remain unclear. Resistance to OA has been described either as P-gp-dependent (Chambers, Raynor & Kuo 1993) or P-gp-independent (Nishio *et al.* 1990), and OA induced a P-gp overexpression together with a decrease in topoisomerase II expression (Tohda *et al.* 1994). However, multidrug-resistance is a complex phenomenon and other mechanisms could be implicated as well. For example, a role for *bcl2* could not be ruled out as (i) OA induced apoptosis by *bcl2*-downregulation (Riordan *et al.* 1998), (ii) *bcl2* gene transfer prevented apoptosis induced by OA (Benito *et al.* 1997, Lerga *et al.* 1999), and (iii) drug-resistant cells could constitutively overexpress the *bcl2* protein and mRNA (Palissot *et al.* 1998).

ACKNOWLEDGEMENTS

The financial support of the 'Comités Départementaux de la Haute-Marne, de l'Aube et de l'Aisne de la Ligue contre le Cancer' is gratefully acknowledged. S. Yatouji is the recipient of a fellowship from the Région Champagne-Ardenne.

REFERENCES

- AJIRO K, YODA K, UTSUMI K, NISHIKAWA Y. (1996) Alteration of cell cycle-dependent histone phosphorylations by okadaic acid. Induction of mitosis-specific H3 phosphorylation and chromatin condensation in mammalian interphase cells. *J. Biol. Chem.* **271**, 13197.
- BARTELS PH, DA SILVA VD, MONTIRONI R *et al.* (1998) Chromatin texture signatures in nuclei from prostate lesions. *Anal. Quant. Cytol. Histol.* **20**, 407.
- BENITO A, LERGA A, SILVA M *et al.* (1997) Apoptosis of human myeloid leukemia cells induced by an inhibitor of protein phosphatases (okadaic acid) is prevented by Bcl-2 and Bcl-X (L). *Leukemia* **11**, 940.
- CHADEE DN, TAYLOR WR, HURTA RA *et al.* (1995) Increased phosphorylation of histone H1 in mouse fibroblasts transformed with oncogenes or constitutively active mitogen-activated protein kinase kinase. *J. Biol. Chem.* **270**, 20098.
- CHAMBERS TC, RAYNOR RL, KUO JF. (1993) Multidrug-resistant human KB carcinoma cells are highly resistant to the protein phosphatase inhibitors okadaic acid and calyculin A. Analysis of potential mechanisms involved in toxin resistance. *Int. J. Cancer* **53**, 323.
- COLOMB E, DUSSERT C, MARTIN PM. (1991) Nuclear texture parameters as discriminant factors in cell cycle and drug sensitivity studies. *Cytometry* **12**, 15.
- DELIGDISCH L, MIRANDA C, BARBA J, GIL J. (1993) Ovarian dysplasia: nuclear texture analysis. *Cancer* **72**, 3253.
- DÖRMER P, ABMAYR W. (1979) Correlation between nuclear morphology and rate of deoxyribonucleic acid synthesis in a normal cell line. *J. Histochem. Cytochem.* **27**, 188.
- DÖRMER P, ABMAYR W, GIARETTI W. (1984) Correlation between protein synthesis rate and nuclear morphology of human erythroblasts as determined by quantitative autoradiography and high-resolution image analysis. *Cytometry* **5**, 131.
- DOUDKINE A, MACAULAY C, POULIN N, PALCIC B. (1995) Nuclear texture measurements in image cytometry. *Pathologica* **87**, 286.
- DUFER J, BIAKOU D, JOLY P *et al.* (1989) Quantitative morphological aspects of granulocytic differentiation induced in HL-60 cells by dimethylsulfoxide and retinoic acid. *Leuk. Res.* **13**, 621.
- DUFER J, LIAUTAUD-ROGER F, BARBARIN D, CONINX P. (1993) Nucleus image analysis as a possible prognostic tool in grading breast cancer. *Biomed. Pharmacother.* **47**, 131.
- DUFER J, MILLOT-BROGLIO C, OUM HAMED Z *et al.* (1995) Nuclear DNA content and chromatin texture in multidrug-resistant human leukemic cell lines. *Int. J. Cancer* **60**, 108.
- FENG JL, IRVING J, VILLEPONTEAU B. (1991) A phosphatase inhibitor enhances the DNase I sensitivity of active chromatin. *Biochemistry* **30**, 4747.
- FERLINI C, BISELLI R, SCAMBIA G, FATTOROSI A. (1996) Probing chromatin structure in the early phases of apoptosis. *Cell Prolif.* **29**, 427.
- FISCHER AH, CHADEE DN, WRIGHT JA *et al.* (1998) Ras-associated nuclear structural change appears functionally significant and independent of the mitotic signaling pathway. *J. Cell Biochem.* **70**, 130.
- GALLOWAY MM. (1975) Texture analysis using gray-level run lengths. *Comp Graph Image Proc.* **4**, 172.
- GIROUD F. (1987). *Approches Cytométriques de la Prolifération et de la Différenciation: Notion de Facies Chromatinien*. Grenoble, University of Grenoble.
- GIROUD F, GAUVAIN C, SEIGNEURIN D, VON HAGEN V. (1988) Chromatin texture changes related to proliferation and maturation in erythrocytes. *Cytometry* **9**, 339.
- GORISSE MC, CARPENTIER Y, JOLY P *et al.* (1990) Alterations of growth fraction and DNA content in K562 cells by differentiating agents. *Cytometry* **11**, 888.
- HAMILTON PW, BARTELS PH, WILSON RH, SLOAN JM. (1995) Nuclear texture measurements in normal colorectal glands. *Anal. Quant. Cytol. Histol.* **17**, 397.
- HARALICK RM, SHANMUGAN K, DINSTEN I. (1973) Textural features for image classification. *IEEE Trans. Syst. Man. Cyber. SMC* **3**, 610.
- JENSEN PH, FLADMARK KE, GJERTSEN BT, VINTERMYR OK. (1999) Caspase I-related protease inhibition retards

- the execution of okadaic acid- and camptothecin-induced apoptosis and PAI-2 cleavage, but not commitment to cell death in HL-60 cells. *Br. J. Cancer* **79**, 1685.
- JORGENSEN T, YOGESAN K, TVETER KJ *et al.* (1996) Nuclear texture analysis: a new prognostic tool in metastatic prostate cancer. *Cytometry* **24**, 277.
- LERGA A, RICHARD C, DELGADO MD *et al.* (1999) Apoptosis and mitotic arrest are two independent effects of the protein phosphatases inhibitor okadaic acid in K562 leukemia cells. *Biochem. Biophys. Res. Commun.* **260**, 256.
- LIAUTAUD-ROGER F, TEYSSIER JR, FERRE D *et al.* (1992) Can chromatin texture predict structural karyotypic changes in diploid cells from thyroid cold nodules? *Anal. Cell. Pathol.* **4**, 421.
- MELLO ML, CONTENTE S, VIDAL BC *et al.* (1995) Modulation of ras transformation affecting chromatin supraorganization as assessed by image analysis. *Exp. Cell Res.* **220**, 374.
- NISHIO K, SUGIMOTO Y, NAKAGAWA K *et al.* (1990) Cross-resistance to tumour promoters in human cancer cell lines resistant to adriamycin or cisplatin. *Br. J. Cancer* **62**, 415.
- PALISSOT V, BELHOUSSE R, CARPENTIER Y *et al.* (1998) Resistance to apoptosis induced by topoisomerase I inhibitors in multidrug-resistant HL60 leukemic cells. *Biochem. Biophys. Res. Commun.* **245**, 918.
- PALISSOT V, LIAUTAUD-ROGER F, CARPENTIER Y, DUFER J. (1996a) Analysis of DNA content in multidrug-resistant cells by image and flow cytometry. *Cell Prolif.* **29**, 549.
- PALISSOT V, LIAUTAUD-ROGER F, CARPENTIER Y, DUFER J. (1996b) Image cytometry of early nuclear events during apoptosis induced by camptothecin in HL-60 leukemic cells. *Cytometry* **25**, 341.
- RIEBER MS, RIEBER M. (1992) Accessibility to DNA in carcinoma chromatin is promoted by nanomolar okadaic acid: effect on AT-rich DNA binding proteins. *Cancer Res.* **52**, 6397.
- RIORDAN FA, FORONI L, HOFFBRAND AV *et al.* (1998) Okadaic acid-induced apoptosis of HL60 leukemia cells is preceded by destabilization of bcl-2 mRNA and downregulation of bcl-2 protein. *FEBS Lett.* **435**, 195.
- ROGER R, ISAAD C, PALLARDY M *et al.* (1996) BCR-ABL does not prevent apoptotic death induced by human natural killer or lymphokine-activated killer cells. *Blood* **87**, 1113.
- SANTISTEBAN MS, BRUGAL G. (1995) Fluorescence image analysis of the MCF-7 cycle related changes in chromatin texture. Differences between AT- and GC-rich chromatin. *Anal. Cell. Pathol.* **9**, 13.
- SCHONTHAL AH. (1995) Regulation of gene expression by serine/threonine protein phosphatases. *Semin. Cancer Biol.* **6**, 239.
- SMITH ML, BORTNICK RA, SHEIKH MS, FORNACE AJ Jr. (1998) Chromatin relaxation by overexpression of mutant p53, HPV16-E6, or cyclin G transgenes. *Exp. Cell Res.* **242**, 235.
- TAYLOR WR, CHADEE DN, ALLIS CD *et al.* (1995) Fibroblasts transformed by combinations of ras, myc and mutant p53 exhibit increased phosphorylation of histone H1 that is independent of metastatic potential. *FEBS Lett.* **377**, 51.
- TOHDA H, YASUI A, YASUMOTO T *et al.* (1994) Chinese hamster ovary cells resistant to okadaic acid express a multidrug resistant phenotype. *Biochem. Biophys. Res. Commun.* **203**, 1210.
- VAN VELTHOVEN R, PETEIN M, OOSTERLINCK WJ *et al.* (1995) The use of digital image analysis of chromatin texture in Feulgen-stained nuclei to predict recurrence of low grade superficial transitional cell carcinoma of the bladder. *Cancer* **75**, 560.
- VIDAL BC, RUSSO J, MELLO MLS. (1998) DNA content and chromatin texture of Benzo[a]pyrene-transformed human breast epithelial cells as assessed by image analysis. *Exp. Cell Res.* **244**, 77.
- WEYN B, VAN DE WOUWER G, VAN DAELE A *et al.* (1998) Automated breast tumor diagnosis and grading based on wavelet chromatin texture description. *Cytometry* **33**, 32.